

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claims 1, 2, 8-15 have been amended as follows:

1.(Amended) A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

- (a) preparing a probe A and a probe B, said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a binding molecule bound to the sequence F', and
said probe B being a second probe which has a sequence S' complementary to a second partial sequence S of the target nucleic acid and a flag bound to the sequence S',
where said flag is a double-stranded sequence and has a marker substance in one of the double strand;
- (b) hybridizing the first probe A with the first partial sequence F of the target nucleic acid and hybridizing the second probe B with the second partial sequence S of the target nucleic acid;
- (c) ligating the first probe A and the second probe B both being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);
- (d) binding the binding molecule to a substance ~~capable of being~~ which is paired up therewith, thereby recovering the probe (A+B); and
- (e) recovering a single-stranded nucleic acid having the marker substance of the double stranded nucleic acid constituting the flag and detecting or quantifying the marker substance, thereby detecting or quantifying the target nucleic acid in the specimen.

2.(Amended) A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of the sequences F1'-Fn', and

said probes B1-Bn (n is an integer of 2 or more) being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or more) of the target nucleic acids and flags bound to the sequences S1'-Sn', where each of said flags is a double-stranded sequence and has a marker substance in one of the double strand; and

(b) respectively hybridizing the first probes A1-An with the first partial sequence F1-Fn of the target nucleic acids, and simultaneously hybridizing the second probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids, respectively;

(c) respectively ligating the first probes A1-An and the second probes B1-Bn, both being hybridized with the target nucleic acids, respectively, thereby obtaining probes (A1+B1)-(An+Bn) (n is an integer of 2 or more);

(d) binding the binding molecule to a substance ~~capable of being~~ which is paired up therewith, thereby recovering the probes (A1+B1)-(An+Bn); and

(e) recovering a single-stranded nucleic acid having the marker substance from the double-stranded nucleic acid constituting each of the flags and detecting or quantifying the marker substance, thereby detecting or quantifying each of the target nucleic acids N1-Nn in the specimen.

8.(Amended) A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

(a) preparing a probe A and a probe B,

said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a binding molecule bound to the sequence F', and

said probe B being a second probe which has a sequence S' complementary to a second partial sequence S of the target nucleic acid and a flag sequence FL consisting of 4 units bound to the sequence S', where said flag FL sequence hybridizes with a sequence FL' bound to the sequence S' to form a double-stranded sequence; and

(b) mixing the probe A, probe B and the specimen, thereby hybridizing the probe A with the first partial sequence F of the target nucleic acid, and simultaneously hybridizing the second probe B with the second partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B, both being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);

(d) binding the binding molecule to a substance ~~capable of being~~ which is paired up therewith, thereby recovering the probe (A+B); and

(e) denaturing the double-stranded flag sequence of the probes (A+B) recovered into single-stranded flag sequence;

(f) hybridizing the single-stranded flag sequence with two primers one of which has a binding molecule B and the other of which has a marker substance L, and extending the primers to form a complementary strand of the flag sequence FL, thereby obtaining a double strand;

(g) binding a binding molecule B with a substance capable of being paired with the binding molecule B, thereby recovering the double strand; and

(h) detecting or quantifying the target substance L, thereby detecting or quantifying the target nucleic acid in the specimen.

9.(Amended) A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of the sequences F1'-Fn', and

said probes B1-Bn (n is an integer of 2 or more) being second probes which respectively have sequences S1-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or more) of the target nucleic acids, and flag sequences FL1-FLn each consisting of 4 units, bound to the sequences S1'-Sn', where said flag sequences FL1-FLn hybridize respectively with sequences FL1'-FLn' bound to the sequences S1'-Sn' to form double-stranded sequences; and

- (b) mixing the probes A1-An, the probes B1-Bn, and the specimen, thereby hybridizing probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn, and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn;
- (c) respectively ligating the probes A1-An and the probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes (A1+B1)-(An+Bn);
- (d) binding each of the binding molecules to a substance ~~capable of being~~ which is paired up therewith, thereby recovering the probes (A1+B1)-(An+Bn); and
- (e) denaturing the double-stranded flag sequences of the probes (A1+B1)-(An+Bn) recovered into single-stranded flag sequences;
- (f) hybridizing the single-stranded flag sequences FL1-FLn with two primers one of which has a binding molecule B and the other of which has a marker substance L, and extending the two primers, to form complementary strands of the flag sequences FL1-FLn, thereby obtaining double strands;
- (g) binding a binding molecule B with a substance capable of being paired therewith, thereby recovering the double strands; and
- (h) detecting or quantifying the marker substance L, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen.

10.(Amended) A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

- (a) preparing a probe A and a probe B,

said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a binding molecule bound to the sequence F', and

said probe B being a second probe which has a sequence S' complementary to a second partial sequence S of the target nucleic acid and a flag consisting of 4 units bound to the sequence S', where said flag FL is a double-stranded sequence; and

(b) mixing the probe A, the probe B, and the specimen, thereby hybridizing the probe A with the first partial sequence F of the target nucleic acid and simultaneously hybridizing the probe B with the second partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B, both being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);

(d) binding the binding molecule to a substance ~~capable of being~~ which is paired up therewith, thereby recovering the probe (A+B); and

(e) denaturing the double-stranded nucleic acid constituting the flag into single-stranded nucleic acid;

(f) amplifying the single-stranded nucleic acid present in a liquid phase by PCR, thereby performing an encode reaction;

(g) performing transcription of a sequence FL' complementary to the single stranded flag sequence obtained by the encode reaction, by use of two primers one of which is a primer having another binding molecule and the other of which is a primer having a marker substance, thereby performing a decode reaction;

(h) binding said another binding molecule to a substance being paired up therewith, recovering a nucleic acid molecule obtained by the decode reaction; and

(i) detecting or quantifying the marker substance, thereby detecting or quantifying the target nucleic acid.

11.(Amended) A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of the sequences F1'-Fn', and

said probes B1-Bn (n is an integer of 2 or more) being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or more) of the target nucleic acids and flag sequences FL1-FLn each consisting of 4 units, bound to the sequences S1'-Sn';

(b) mixing the first probes A1-An, the second probes B1-Bn, and the specimen, thereby hybridizing the probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn, respectively;

(c) respectively ligating the probes A1-An and the probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes (A1+B1)-(An+Bn) (n is an integer of 2 or more);

(d) binding the binding molecule to a substance ~~capable of being~~ which is paired up therewith, to recover the probes (A1+B1)-(An+Bn), and thereafter performing an encode reaction of each of the flags FL1-FLn; and

(e) performing a decode reaction of the sequences FL1'-FLn' complementary to the flags FL1-FLn obtained by the encode reaction; and

(f) detecting or quantifying the nucleic acid molecules obtained by the decode reaction, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen.

12.(Amended) A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of the sequences F1'-Fn', and

said probes B1-Bn (n is an integer of 2 or more) being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or more) of the target nucleic acids and flag sequences FL1-FLn each consisting of 4 units, bound to the sequences S1'-Sn', respectively,

(b) mixing the probes A1-An, the probes B1-Bn, and the specimen, thereby hybridizing probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn, and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn, respectively;

- (c) respectively ligating the probes A1-An and the probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes (A1+B1)-(An+Bn);
- (d) binding each of the binding molecules to a substance ~~capable of being~~ which is paired up therewith to recover the probes (A1+B1)-(An+Bn), and thereafter performing an encode reaction for each of the flags FL1-FLn; and
- (e) performing a decode reaction of the sequences F11'-FLn' complementary to the flags FL1-FLn (n is an integer of 2 or more) obtained by the encode reaction; and
- (h) detecting the nucleic acid molecules obtained by the decode reaction, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen,
- wherein 2 units of 4 units are sequences functioning as primers for PCR amplification.

13.(Amended) A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

- (a) preparing a probe A and a probe B, said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a binding molecule bound to the sequence F', and
- said probe B being a second probe which has a sequence S' complementary to a second partial sequence S of the target nucleic acid and a flag consisting of 4 units bound to the sequence S1, where said flag FL is a double-stranded sequence and said 4 units consist of SD, D0, D1 and ED each having an arbitrary sequence, ~~bounded~~ bound to each other sequentially in the order mentioned; and
- (b) mixing the probe A, the probe B, and the specimen, thereby hybridizing the probe A with the first partial sequence F of the target nucleic acid and simultaneously hybridizing the probe B with the second partial sequence S of the target nucleic acid;

- (c) ligating the probe A and the probe B both being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);
- (d) binding the binding molecule to a substance ~~capable of being~~ which is paired up therewith, thereby recovering the probe (A+B); and
- (e) denaturing the double-stranded nucleic acid constituting the flag into a single-stranded nucleic acid;
- (f) hybridizing the single-stranded nucleic acid obtained in a liquid phase with sequences complementary to sequences D11-D1n labeled with a marker substance, as primers,
- (g) extending the primers hybridized
- (h) denaturing a double-stranded nucleic acid having primers into a single-stranded nucleic acid;
- (i) hybridizing the sequences D01-D0n specifically with the primers extended to detect or quantify the marker substances included in the sequences D01-D0n, thereby detecting or quantifying the target nucleic acids.

14.(Amended) The method according to claims 10 to 12, wherein the decode reaction comprises, where said flag(s) FL is a double-stranded sequence and said 4 units consist of SD, D0, D1 and ED each having an arbitrary sequence, bound to each other sequentially in the order mentioned,

- (i) performing PCR for a single-strand sequence encoded using SD sequence to which a binding molecule is attached, and ED sequence, as primers;
- (ii) binding a binding molecule bound to the SD sequence to a substance ~~capable of being~~ which is paired up therewith, thereby recovering a PCR product;

- (iii) denaturing the PCR produce into a single strand
- (iv) hybridizing the single strand with primers D11'-D1n' labeled;
- (v) extending the primers'
- (vi) denaturing the primers extended into single strands;
- (vii) hybridizing extended single strands of the primers with sequences D01-D0n to detect or quantify marker substances included in that sequences D01-D0n, thereby detecting or quantifying the target nucleic acid.

15.(Amended) The method according to claims 10 to 12, wherein the decode reaction comprises, where said flag FL is a double-stranded sequence and said 4 units consist of SD, D0, D1 and ED each having an arbitrary sequence, bound to each other sequentially in the order mentioned; and

- (i) performing PCR for a single-stranded sequence encoded using SD sequence to which a binding molecule is attached and ED sequence, as primers;
- (ii) binding the binding molecule bound to the SD sequence to a substance ~~capable of being~~ which is paired up therewith, thereby recovering a PCR product;
- (iii) denaturing the PCR product into a single strand;
- (iv) mixing the sequences D1n' labeled and D0n' labeled, thereby hybridizing the single strand with the sequences D1n' and D0n';
- (v) denaturing the sequences ligated into a single-stranded sequence;
- (vii) hybridizing sequences D01-D0n with the single-stranded sequence labeled with a marker substance, to detect or quantify the marker substance, thereby detecting or quantifying the target nucleic acid.